

Investigation of the Prevalence of *Dirofilaria immitis* in Dogs in Ardahan RegionCemalettin Ayvazoglu^{1,a,*}, Enes Akyüz^{2,b}, Metin Ögün^{3,c}, Pınar Ayvazoglu Demir^{4,d}, Şemiştan Kiziltepe^{5,e}¹Ardahan University, Nihat Delibalta Göle Vocational High School, Ardahan, Türkiye²Kafkas University, Faculty of Veterinary Medicine, Department of Veterinary Internal Medicine, Kars, Türkiye³Kafkas University, Faculty of Art and Science, Department of Chemistry, Kars, Türkiye⁴Kafkas University, Faculty of Veterinary Medicine, Department of Livestock Economics, Kars, Türkiye⁵Iğdır University, Tuzluca Vocational School, Iğdır, Türkiye^aORCID: 0000-0003-2064-0657; ^bORCID: 0000-0002-3288-2058; ^cORCID: 0000-0002-2599-8589;^dORCID: 0000-0002-7010-0475; ^eORCID: 0000-0003-3727-8893

*Corresponding Author

E-mail: cemayvazoglu@hotmail.com

Received: March 07, 2022

Accepted: April 18, 2022

Abstract

The aim of this study was to determine the prevalence of *Dirofilaria immitis* (*D. immitis*) in dogs in the Ardahan region. The study material consisted of 100 Akbaş crossbred dogs (53 females and 47 males) between the ages of 3-7 in Ardahan region. An immunochromatographic analysis test kit was used to determine seroprevalence. The presence of antigen against *D. immitis* was determined as 12% (12/100). When the dogs with antigens against *D. immitis* were evaluated according to their age, it was determined that the highest positivity was in 4 years old (15%) ($P>0.05$). Antigen presence against *D. immitis* was detected in 10.6% of male dogs and 13.2% of female dogs ($P>0.05$). According to the data obtained from this study, it was concluded that *D. immitis* was seen in dogs in the Ardahan region and that protection and control measures should be taken for the eradication of this disease due to reasons such as global warming, wildlife and lack of education.

Keywords: Ardahan, canine vector-borne diseases, dirofilariasis, dog.**INTRODUCTION**

Vectors and vector-borne diseases; Due to factors such as climate changes, resistance to unconsciously used drugs, genetic changes in pathogenic factors, uncontrollable human and animal movements, they are constantly kept up-to-date because they are seen at increasing rates day by day (Shaw et al., 2001). *Dirofilaria immitis* (*D. immitis*), which is common in the world, is one of the most important vector-borne agents infecting dogs among nematode species (Day, 2011).

Dirofilaria immitis; It is a parasitic zoonosis in the superfamily Filaroidea of the nematode class, biologically transmitted by intermediate host mosquitoes (Anopheles, Aedes, Culex, Taeniorhynchus, Mansonia and Armigenes), whose final hosts are carnivores (dog, cat, fox, etc.) and humans (Taşçı and Kılıç, 2012; Yabaneri et al., 2017). The agent can cause serious pathological disorders and even death by localizing in the right atrium and ventricle of the heart, pulmonary arteries, camera oculi anterior in animals and humans (Venco, 2007). Clinically, symptoms such as respiratory distress, dry and hoarse cough, fatigue, ascites, and nosebleeds are observed even after short-distance running (Taşçı and Kılıç, 2012).

Recognizing the presence and prevalence of vector-borne pathogens in dogs; It is necessary to establish the treatment protocol and to determine the prevention and control measures (Irwin, 2009). In different studies conducted in this context, it was determined that the prevalence of *D. immitis* decreased from 30% to 19% in

Spain and from 46% to 23% in Japan due to the increasing awareness of veterinarians and animal owners (Montoya-Alonso et al., 2010; Oi et al., 2014). In the literature review, no study was found to determine the seroprevalence of *D. immitis* in dogs in the Ardahan region of Turkey. Therefore, in the presented study, it was aimed to determine the seroprevalence of *D. immitis* in dogs in the Ardahan region of Turkey with a rapid test kit.

MATERIALS AND METHODS**Ethical approve**

The study was conducted with the decision of Ardahan University Scientific Publication and Ethics Committee dated 04.03.2021 and numbered E-67796128-000-2100006252.

Animal material

In this study, the animal material was collected from Akbaş crossbred dogs in Ardahan region of different ages and genders; a total of 100 dogs, who were found to be healthy as a result of clinical examination, were between 3-7 years of age, whose general condition, interest and appetite towards the environment were normal, and no antiparasitic treatment had been applied before. Dogs older than 1 year were used in the study as microfilaria occurs on average 6 months after infective larvae enter the host and the number of microfilariae in the blood increases within 6 months thereafter (Calvert and Rawlings, 1985).

Serological analysis

The presence of antigens against *D. immitis* in blood samples was evaluated with a rapid test kit with high sensitivity and specificity, working with the principle of immunochromatographic analysis (SensPERT® One Step Rapid Test Kit, VetAll Laboratories, Korea). The test kit was used in accordance with the manufacturer's recommendations. In order to determine the presence of *D. immitis* antigens, 1 drop of sample 2 drip diluent was dripped into the relevant chamber in the kit through a disposable dropper from blood serum samples obtained from dogs in the Ardahan region between 15 July and 1 September 2021. Then, the rapid test kit result was evaluated within 5-10 minutes. Those with control and test lines in the area beyond the chamber were evaluated as positive, and those with only control lines were evaluated as negative.

Statistical analysis

SPSS 20 package program was used for statistical analysis of the obtained data. Chi-square test was used to compare the incidence of *D. immitis* in dogs according to age and gender.

RESULTS

In the study, 100 Akbaş crossbred dogs were used. 32 of these animals were selected by random sampling from Ardahan centre, 15 Çıldır, 18 Göle, 15 Damal, 10 Hanak and 10 Posof and its affiliated villages.

Within the scope of the study, the blood serum samples of 100 dogs of different ages and genders without any signs of disease after clinical examination from 100 different foci were examined with the rapid test kit and as a result of the analysis, it was determined that *D. immitis* was positive in 12% of the dogs, while it was negative in 88%.

In the analysis, it was determined that 25% of the dogs used in the study were under the age of 3, 40% were 4 years old, and 35% were 5 years and older. The incidence of *D. immitis* by age groups is given in Table 1.

Table 1. *D. immitis* incidence rate by age of dogs

Age	<i>D. immitis</i>		Total
	Negative	Positive	
≥3	23	2	25
	92.0%	8.0%	100.0%
4	34	6	40
	85.0%	15.0%	100.0%
5≤	31	4	35
	88.6%	11.4%	100.0%
Total	88	12	100
	88.0%	12.0%	100.0%

In the analysis, *D. immitis* was positive in 8% of dogs aged 3 years and younger, 15% of dogs aged 4 years and 11.4% of dogs aged 5 years and older, and the incidence of *D. immitis* with the age of the animals in the chi-square analysis. No statistically significant relationship was found between ($P>0,05$).

In the study, it was determined that 53% of the dogs whose blood samples were taken were female and 47% were male, and the gender of the animals and the incidence of *D. immitis* are given in Table 2.

Table 2. *D. immitis* incidence rate by gender

Gender	<i>D.immitis</i>		Total
	Negative	Positive	
Female	46	7	53
	86.8%	13.2%	100.0%
Male	42	5	47
	89.4%	10.6%	100.0%
Total	88	12	100
	88.0%	12.0%	100.0%

$\chi^2=0.156$ $P=0.693$ $P>0.05$

In the study, *D. immitis* was positive in 13.2% of the female dogs and 10.6% of the male dogs, and no statistically significant relationship was found between the gender of the animals and the incidence of *D. immitis* in the chi-square analysis ($P>0.05$).

DISCUSSION AND CONCLUSION

Filarial nematodes that settle on dogs, due to both the diseases they cause and their zoonotic properties; It has an increasing importance due to factors such as climate changes, resistance to unconsciously used drugs, genetic changes in pathogenic factors, uncontrolled human and animal movements (Shaw et al., 2001). Therefore, knowing the presence and prevalence of vector-borne pathogens is essential for establishing a treatment protocol and determining prevention and control measures (Irwin, 2009). In this context, too; The seroprevalence of *D. immitis*, which is transmitted by mosquitoes, is one of the most researched diseases all over the world.

D. immitis is reported to be still endemic in the south eastern regions of Europe, Africa and Asia (McCall et al., 2008). According to the studies, the prevalence of *D. immitis* in the world is presented in Table 3.

Table 3. The prevalence of *D. immitis* in the world in different studies.

Line No	Country	Prevalence	Reference
1	Spain	3%	Montoya-Alonso et al., 2017
2	Portugal	4%	Alho et al., 2018
3	Germany	1.4%	Vrhovec et al., 2017
4	Croatia	0,4%	Jurković et al., 2019
5	Greece	25%	Diakou et al., 2019
6	Romania	14%	Mircean et al., 2012
7	Bulgaria	40%	Morchón et al., 2018
8	Iranian	53.8%	Khedri et al., 2014
9	Türkiye	18%	Köse and Erdoğan, 2012
10	Japan	23%	Oi et al., 2014

In different studies, it has been reported that the highest prevalence of *D. immitis* is seen in Iran with 53.8% and the lowest in Croatia (Table 3). In studies conducted in different years in Turkey, the prevalence of *D. immitis* was found to be positivity at different rates between 0-40% according to the provinces (Table 4). Possible reasons for this situation are thought to be environmental and climatic conditions, season, number of animals studied, density of vector population, diagnostic methods and status of infection.

Table 4 According to different studies, the prevalence of *D. immitis* in provinces in Turkey

Line No	Province	Prevalence	Reference
1	Afyon-Eskişehir	2.5%	Kozan et al., 2007
2	Nevşehir	2.17%	Yabaneri et al., 2017
3	Burdur	22%	Adanır et al., 2013
4	Diyarbakır	2.4%	İcen et al. 2011
5	Ankara	9.3%	Öge et al., 2003
6	Hatay	26%	Yaman et al., 2009
7	Iğdır	40%	Sarı et al., 2013
8	Kırıkkale	5.8%	Yıldız et al., 2008
9	Kayseri	9.6%	Yıldırım et al., 2007
10	Sivas	2.9%	Ataş et al., 2018
11	Van	17.8%	Yaşar et al., 2007
12	İstanbul	1.52%	Öncel and Vural, 2005
13	Elazığ	1.8%	Şimşek and Çiftci, 2016
14	Kars	8.8%	Taşcı and Kılıç, 2012
15	Antalya	0%	Küçükler and Şahinduran, 2018
16	Osmaniye	1%	Gökmen and ark., 2019
17	Siirt	0%	Çelik et al., 2020
18	Samsun	0%	Çakıroğlu and Meral, 2007
19	Erzurum	4.4%	Demir and Aktaş, 2020
20	İzmir	0%	Ertekin, 2017

In different studies stated that, there was no difference between males and females in the evaluation of the presence of *D. immitis* in dogs in terms of gender difference (Güven et al., 2017; Ataş et al., 2018), although seropositivity was higher in males, this level was not statistically significant (İcen et al. 2011; Adanır et al., 2013) seropositivity is high in males and this height is statistically significant (Şimşek et al., 2011; Demir and Aktaş, 2020) or that seropositivity is higher in females, but this height is not statistically significant, there are studies that reveal this (Yaman et al., 2009; Sarı et al., 2013). In our study, there was no relationship between the gender difference and the incidence of *D. immitis*.

When the presence of *D. immitis* in dogs is evaluated according to age, the prevalence does not change according to age (MH Razi and Ar, 2010; Güven et al., 2017); Seropositivity increases with increasing age, but this increase is not statistically significant (Yıldırım et al., 2007; Çetinkaya et al. 2016; Demir and Aktaş, 2020), or seropositivity increases with increasing age and this increase is statistically significant (Sarı et al., 2013; Adanır et al., 2013) have been reported. When the data obtained in our study were evaluated according to age, it was determined that there was no statistically significant difference between age groups.

As a result, with the increasing awareness and preventive medicine studies as a result of the studies, *D. immitis* (Genchi and Kramer, 2020), which decreased in Europe; It has been concluded that it is seen in dogs in the Ardahan region and that protection and control measures should be taken for the eradication of this disease due to reasons such as global warming, wildlife, lack of education.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: C.A., Design: C.A., G.G., Data Collection or Processing: C.A., E.A., M.Ö., Ş.K., Analysis or

Interpretation: C.A., P.A.D., Literature Search: C.A., Writing: C.A., E.A., M.Ö., P.A.D., G.G., Ş.K.

Financial Support

This study was funded by Ardahan University Scientific Research Projects Coordinator with the project number ARÜ-BAP/2021-002.

REFERENCES

- Adanır R, Sezer K, Onur K. 2013. The prevalence of *Dirofilaria immitis* in dogs with different breed, ages and sex. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 60(4): 241-244.
- Alho AM, Meireles J, Schnyder M, Cardoso L, Belo S, Deplazes P, de Carvalho LM. 2018. *Dirofilaria immitis* and *Angiostrongylus vasorum*: the current situation of two major canine heartworms in Portugal. Veterinary Parasitology, 252: 120-126.
- Ataş AD, Altay K, Alim A, Özkan E. 2018. Survey of *Dirofilaria immitis* in dogs from Sivas Province in the Central Anatolia Region of Turkey. Turkish Journal of Veterinary and Animal Sciences, 42(2): 130-134.
- Calvert CA, Rawlings CA. 1985. Pulmonary manifestations of heartworm disease. Veterinary Clinics of North America: Small Animal Practice, 15(5): 991-1009.
- Cetinkaya H, Akyazi I, Ozkurt M, Matur E. 2016. The serologic and molecular prevalence of heartworm disease in shelter dogs in the Thrace Region of Turkey. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 22(5): 751-755.
- Çakıroğlu D, Meral Y. 2007. Investigation of *Dirofilaria immitis* infestation in dogs in Samsun Region. Journal of Istanbul Veterinary Sciences, (2): 1-12.
- Çelik ÖY, Çelik ÖY, Şahin T, İrak K, Bolacalı M, Biçici Ö, Baldaz V. 2020. A Serologic Survey of *Dirofilariasis*, *Leishmaniasis*, *Ehrlichiosis*, and *Anaplasmosis* in Dogs in Siirt Province. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi, 34(2): 103-108.
- Day MJ. 2011. The immunopathology of canine vector-borne diseases. Parasites & Vectors, 4(1): 1-13.
- Demir A, Aktaş MS. 2020. Erzurum Yöresindeki Köpeklerde *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* ve *Anaplasma* spp Seroprevalansının Araştırılması. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi, 34(2): 91-96.
- Diakou A, Soubasis N, Chochlios T, Oikonomidis IL, Tselekis D, Koutinas C, Rallis, T. 2019. Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece. Parasitology research, 118(2): 677-682.
- Ertekin B. 2017. İzmir hayvan barınaklarındaki köpeklerde *Dirofilaria immitis*'in prevalansı. Master's thesis, Sağlık Bilimleri Enstitüsü.
- Genchi C, Kramer LH. 2020. The prevalence of *Dirofilaria immitis* and *D. repens* in the Old World. Veterinary Parasitology, 280: 108995.
- Gokmen TG, Günaydın E, Turut N, Bünyamin A, Özgür K, Ütük AE. 2019. A serosurvey on some canine vector-borne zoonoses (*Anaplasma* spp., *Ehrlichia* spp., *Borrelia burgdorferi*, *Dirofilaria immitis* and *Leishmania* spp.) in Osmaniye. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 14(2): 151-158.
- Güven E, Avcioğlu H, Cengiz S, Hayırlı A. 2017. Vector-borne pathogens in stray dogs in Northeastern Turkey. Vector-Borne and Zoonotic Diseases, 17(8): 610-617.

- Hoch H, Strickland K. 2008. Canine and feline dirofilariasis: prophylaxis, treatment, and complications of treatment. *Compendium*, 30(3): 146.
- Irwin PJ. 2009. Canine babesiosis: from molecular taxonomy to control. *Parasites & vectors*, 2(1): 1-9.
- İçen H, Sekin S, Simsek A, Kochan A, Celik OY, Altas MG. 2011. Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* infection in dogs from Diyarbakir in Turkey. *Asian Journal of Animal and Veterinary Advances*, 6(4): 371-378.
- Jurković D, Beck A, Huber D, Mihaljević Ž, Polkinghorne A, Martinković F, Beck R. 2019. Seroprevalence of vector-borne pathogens in dogs from Croatia. *Parasitology research*, 118(1): 347-352.
- Khedri J, Radfar MH, Borji H, Azizzadeh M, Akhtardanesh B. 2014. Canine heartworm in southeastern of Iran with review of disease distribution. *Iranian journal of parasitology*, 9(4): 560-567.
- Kozan E, Sevimli FK, Birdane FM. 2007. Afyonkarahisar ve Eskişehir İl'lerindeki sokak köpeklerinde *Dirofilaria sp.*'nin yayılışı. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 54(2): 145-150.
- Köse M, Erdoğan M. 2012. Serological screening of canine heartworm (*Dirofilaria immitis*) infections in Turkey. *Berliner und Munchener Tierärztliche Wochenschrift*, 125(11-12): 503-508.
- Küçüker S, Şahinduran Ş. 2018. Antalya İlinde bulunan köpeklerde Dirofilariasis, Borreliozis, Ehrlichiazis ve Anaplazmozis' in hızlı test kitleri ile teşhisi ve insidansı üzerine araştırmalar. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, 13(2): 191-200.
- McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L. 2008. Heartworm disease in animals and humans. *Advances in parasitology*, 66: 193-285.
- MHRazi J, Ar A. 2010. A study on *Dirofilaria immitis* in healthy urban dogs from Ahvaz, Iran.
- Mircean V, Dumitrache MO, Györke A, Pantchev N, Jodies R, Mihalca AD, Cozma V. 2012. Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato*, and *Ehrlichia canis*) in dogs from Romania. *Vector-Borne and Zoonotic Diseases*, 12(7): 595-604.
- Montoya-Alonso JA, Carretón E, Juste MC, Mellado I, Morchón R, Simón F. 2010. Epidemiological survey of canine heartworm disease on the island of Gran Canaria (Canary Islands-Spain) between 2000 and 2008. *Veterinary parasitology*, 173(1-2): 165-168.
- Morchón R, Carretón E, Zueva T, Diosdado A, Sed G, Kartashev V, Simón F. 2018. Proceedings of 6th European *Dirofilaria* and *Angiostrongylus* Days. *Parasites & Vectors*, 11(1): 623.
- Oge H, Doğanay A, Oge S, Yıldırım A. 2003. Prevalence and distribution of *Dirofilaria immitis* in domestic dogs from Ankara and vicinity in Turkey. *DTW. Deutsche Tierärztliche Wochenschrift*, 110(2): 69-72.
- Oi M, Yoshikawa S, Ichikawa Y, Nakagaki K, Matsumoto J, Nogami S. 2014. Prevalence of *Dirofilaria immitis* among shelter dogs in Tokyo, Japan, after a decade: comparison of 1999–2001 and 2009–2011. *Parasite*, 21.
- Öncel T, Vural G. 2005. Seroprevalence of *Dirofilaria immitis* in stray dogs in Istanbul and Izmir. *Turkish Journal of Veterinary and Animal Sciences*, 29(3): 785-789.
- Sarı B, Taşçı GT, Kılıç Y. 2013. Seroprevalence of *Dirofilaria immitis*, *Ehrlichia canis* and *Borrelia burgdorferi* in dogs in Iğdır province, Turkey. *infection*, 49-51.
- Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. 2001. Tick-borne infectious diseases of dogs. *Trends in parasitology*, 17(2): 74-80.
- Simsek S, Ciftci AT. 2016. Serological and molecular detection of *Dirofilaria* species in stray dogs and investigation of *Wolbachia* DNA by PCR in Turkey. *Journal of arthropod-borne diseases*, 10(4): 445-453.
- Simsek S, Ozkanlar Y, Balkaya I, Aktas MS. 2011. Microscopic, serologic and molecular surveys on *Dirofilaria immitis* in stray dogs, Turkey. *Veterinary parasitology*, 183(1-2): 109-113.
- Strickland KN. 1998. Canine and feline caval syndrome. *Clinical techniques in small animal practice*, 13(2): 88-95.
- Tasci GT, Kılıç Y. 2012. Kars ve Iğdır Civarındaki Köpeklerde *Dirofilaria immitis* (Leidy, 1856)'nin Prevalansı ve Potansiyel Vektör Sivrisinek Türleri Üzerine Araştırmalar. *Kafkas Univ Vet Fak Derg*, 18, A29-A34.
- Venco L. 2007. Heartworm (*Dirofilaria immitis*) disease in dogs. *Dirofilaria immitis* and *D. repens* in Dog and Cat and Human Infections, 118-125.
- Vrhovec MG, Pantchev N, Failing K, Bauer C, Travers-Martin N, Zahner H. 2017. Retrospective analysis of canine vector-borne diseases (CVBD) in Germany with emphasis on the endemicity and risk factors of leishmaniosis. *Parasitology Research*, 116(1): 131-144.
- Yaman M, Guzel M, Koltas IS, Demirkazık M, Aktas H. 2009. Prevalence of *Dirofilaria immitis* in dogs from Hatay province, Turkey. *Journal of Helminthology*, 83(3): 255-260.
- Yasar G, Koltas IS, Altug N, Demirkazık M, Yüksek N, Azoğlu Z. 2007. Van Yöresi Köpeklerinde *Dirofilaria immitis*' in Seroprevalansı. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 18(2): 5-8.
- Yıldırım A, İca A, Atalay O, Duzlu O, İnci A. 2007. Prevalence and epidemiological aspects of *Dirofilaria immitis* in dogs from Kayseri Province, Turkey. *Research in veterinary science*, 82(3): 358-363.
- Yıldız K, Duru SY, Yağcı BB., Öcal N, Gazyağcı AN. 2008. The prevalence of *Dirofilaria immitis* in dogs in Kırıkkale. *Türkiye Parazitoloji Dergisi*, 32(3): 225-228.